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INTRACORPUSCULAR CONJUGATION IN THE
MALARIAL PLASMODIA AND ITS SIGNIFICANCE

C.F.Craig

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be done in the final settlement of the questions involved, and in placing the proper relative valuation on points already proved. In this work the clinician and the laboratory man must work in conjunction—neither can attain the end in view independently of the other.

In the light of our present knowledge the following conclusions seem to be justified:

1. The division of mammalian tubercle bacilli into two types, human and bovine, first proposed by Theobald Smith in 1898, has been amply confirmed. These types have cultural, morphologic, and tinctorial characteristics by which they may usually be recognized. The chief point of difference, however, is found in the very much greater pathogenic power of the bovine type. Bovine bacilli are, however, met with which have low pathogenic power.

2. No other species of mammal has been shown to harbor a variety of tubercle bacillus so constant in its characteristics as to justify its classification as a third type.

3. Other species suffering from tuberculosis derive their infection from man or from cattle.

4. The human tubercle bacillus, as a rule, has a low pathogenic power for cattle, but cultures are not infrequently found which are virulent for the bovine race.

5. The bovine tubercle bacillus has the power of invading the human body and producing the lesions of tuberculosis.

6. We are at present unable to state the exact proportion of cases in which bovine tuberculosis is transmitted to man, but in view of the evidence at hand we must regard the disease in cattle as the source of a certain part of human tuberculosis, and any relaxation in our laws and precautions against bovine tuberculosis would be most unwise.

INTRACORPUSCULAR CONJUGATION IN THE MALARIAL PLASMODIA AND ITS SIGNIFICANCE.*

BY

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Introduction.—The method of sexual reproduction of the malarial plasmodia in the mosquito, which has been so brilliantly demonstrated by many investigators, suggests the query as to whether or not an analogous process occurs during the life cycle of the parasites in man. So far as I am aware, but little work has been done in this direction, the only contribution upon the conjugation of the parasites in man being the important papers of Ewing,^{1 2} although the process is mentioned by Mannaberg. Ewing, however, came to the conclusion, from his investigations, that while conjugation of the malaria plasmodia undoubtedly occurred in the red blood-corpuscles of man, it was by no means constant, and probably of no essential importance in the human life cycle of the plasmodia. He observed the

process in the tertian and estivoautumnal varieties of the parasites, but had no opportunity of studying it in the quartan type.

Since reading Ewing's contributions upon this subject I have studied intracorpuseular conjugation in many hundreds of specimens of malarial blood, both in fresh and stained preparations, these studies covering a period of nearly five years, and I believe that I am justified in stating, as the result of this work, that this form of conjugation, far from being rare, is of very frequent occurrence in all the varieties of malarial plasmodia, and that it is of vital importance in their human life cycle.

While I do not believe that the facts warrant the assertion that sporulation cannot occur without previous conjugation, it is without doubt true, that conjugation is essential to the maintenance of malarial infection in man, and that in those instances in which it does not occur, the plasmodia undergo asexual sporulation for a limited time and then perish, thus leading to spontaneous recovery which, as is well known, is frequently observed in malarial infections. These studies also prove that in latent malarial infection conjugation is almost never observed, and therefore we are justified in assuming that the severe acute malarial symptoms observed clinically are only present in those cases in which conjugation is. In other words, autoinfection in malaria can only be maintained when conjugation is present and its disappearance is the explanation, partially at least, of spontaneous recovery or the cessation of the clinical symptoms.

To Ewing belongs the credit of first calling the attention of American observers to this phenomenon, and I shall quote verbatim from his writings in reviewing his work. In describing this process he says³:

In four cases of tertian infection I have encountered appearances which seem to admit of no other explanation than that of conjugation of malarial parasites. In a considerable number of other cases similar appearances were found, but much less frequently. A great many red cells showed double infection with young rings. In many instances these rings were entirely separate, each exhibiting a single large granule of chromatin. Many cells, however, contained two rings which were clearly fused together along one segment of the ring, and two large chromatin granules were then invariably found at different points in the rings. The fused parasites usually differed in appearance. One was a large, delicate ring with a thin bow, and chromatin granules of moderate size, while the other was a coarser body with thickened bow, and exhibiting a large chromatin granule. These differences between the two conjugating parasites could not always be found.

In this early stage Ewing found no evidence of the fusion of the chromatin granules, and therefore of the nuclei. Continuing his description of the process, he says⁴:

On examining the parasites in later stages of development, most of them were found to have lost the ring form, and to have spread out into a large number of threads, with nodal thickenings, variously curled in the red cell. These threads evidently represented the pseudopodia of a very active ameboid stage. The chromatin masses were now subdivided into 10 or 12 granules, but in the majority of the cases the masses were far apart, and showed no tendency to unite. In many cells, however, the ameboid figures were less marked, and the masses of

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chromatin lay side by side, united by a little achromatic substance. Later some parasites were found in which the two groups of chromatin granules lay in immediate apposition, surrounded by achromatic substance.

As will be seen in the description of this process which follows, the bodies described by Ewing as showing the division of the chromatin into several scattered granules, collected into two groups, situated far apart, were really not conjugating parasites, but rather, in all probability, parasites in which conjugation was complete, and the chromatin undergoing division prior to sporulation. Intracorpuseular conjugation is always completed before the appearance of pigment within the parasites, and it is impossible to distinguish parasites in which conjugation has occurred from those in which it is absent, after the beginning of pigment formation. The division of the chromatin into two or more distinct masses of granules in the larger tertian plasmodia is a normal step in the development of the spore stage, and is always seen in the developing parasite whether conjugation has occurred or not. It should not, therefore, be regarded as an evidence of conjugation, and the bodies later described by Ewing as nearly full grown cannot be identified as conjugating organisms, for the same appearances are presented in nonconjugating plasmodia.

Ewing describes, somewhat in detail, certain differences in the conjugating parasites, consisting largely in the relative size of the two organisms and their staining reactions. These differences, even as described by him, are comparatively slight, and he states that they cannot always be recognized. From my own observations I have been unable to confirm his observations as regards any essential difference in the morphology of the conjugating bodies, for while in rare instances, one of the organisms may appear slightly larger than the other, may have a larger mass of chromatin, and stain more intensely, in the vast majority of instances the two conjugating parasites are of exactly the same size, and cannot be distinguished one from the other, in any way. The compact deeply-staining form, which Ewing considers as probably essential to conjugation, and which does not commonly assume the ring shape, is of comparatively infrequent occurrence, and is simply a young parasite which happens to conjugate with one which is in a slightly more advanced stage of growth.

The careful study of intracorpuseular conjugation as it occurs in all the varieties of the plasmodia of malaria will demonstrate that, in practically every instance, no distinction can be made between the two conjugating parasites, and that in the vast majority of cases, conjugation takes place between two distinct ring forms of the same size.

As regards the significance of this process Ewing is undecided. He says⁵:

It would seem that a process so fundamental as the conjugation of individuals, if it occurs at all, ought to be an invariable feature of every active infection, but there is not sufficient evidence on which to base any such claim. The four cases referred to as furnishing numerous clear examples of conjugation were selected on account of the abundance of the conjugating forms, but in many other cases less numerous though equally distinct examples were seen, indicating that the process is of very frequent occurrence. On the other hand, it

must be admitted that the majority of specimens from routine cases fail to show any distinct traces of the process; from which it may be concluded that conjugation is not an essential feature of the growth of the parasite.

In a later contribution he says⁶:

The extent and significance of this form of conjugation it is difficult to determine. In the cases in which it can be profitably studied, parasites are very abundant, and in most cases few indications of the process can be detected. It is, therefore, probably not essential to sporulation, and when parasites are scanty the chances of finding typical examples of conjugating pairs are greatly reduced, but the peripheral blood may not be a complete index of the process in the internal viscera. The large size of the sporulating forms developed from conjugating pairs suggests that this process is intended especially to favor the multiplication of the species in the human host. It seems probable that conjugation occurs in the first generations of the infection, and becomes less frequent as the disease progresses, the infection in the human host thereby tending to limit itself.

In the sections of this contribution treating of the frequency of the occurrence of this process, and its significance, I shall discuss more at length the foregoing statements, but it may be noted in passing that Ewing recognized the probability of the importance of the process in relation to autoinfection, and that its cessation resulted in the limitation of the malarial infection.

In the investigation of this subject it has seemed to me that the following points should be definitely determined, if possible: The morphology of the parasites during intracorpuseular conjugation; the frequency of its occurrence; the variety and clinical types of malaria in which the process occurs most frequently, and the significance of the process.

Material.—As regards the material which has been available for this investigation, I may say that it has consisted of nearly 300 cases of malaria presenting clinical symptoms, observed at the United States Army General Hospital, at San Francisco, Cal., while I was director of the bacteriologic laboratory there, together with over 100 cases of latent malarial infection observed at the same place; a series of 75 cases of latent infection in native Filipino children studied at Camp Stotsenburg, and 52 cases of acute malarial infection observed in soldiers and civilians in and around that post.

Of the acute cases studied at San Francisco almost all of them were in soldiers returning from the Philippines, and were recurrences of old malarial infection, while nearly all the acute cases seen at Camp Stotsenburg were initial attacks. From this data it will be seen that the process of conjugation has been studied in three classes of cases: Initial attacks of malaria, recurrent attacks, and latent infections.

Both fresh and stained preparations of the blood have been used, for while the process is best observed in stained specimens of blood, in a few instances I have been so fortunate as to observe the process of conjugation in the fresh specimen, and for that reason both the fresh and stained blood has been studied. The process of conjugation has been studied in all the varieties of malaria, including the quartan type, but it is seen most often and typically in the estivoautumnal infections.

The staining method which I have found to give the

best results is a slightly modified Wright's method, suggested by Dr. H. R. Oliver, of San Francisco, and the staining solution is prepared and used as follows:

Add .5 gm. of sodium bicarbonate to 100 cc. of distilled water, dissolving thoroughly, and then add 1 gm. of methylene-blue (Grubler's) and heat for an hour in an Arnold sterilizer after the steam is up. After heating, allow the solution to cool. Make a 1 to 1,000 solution of yellow aqueous eosin (Grubler's) and add this while stirring, to the cooled methylene-blue solution, in the proportion of 500 cc. of the eosin solution to 75 cc. of the blue solution. This should be done in a large porcelain dish. After letting the mixture stand for a few moments, filter through a small filter paper and save the residue, which should be dried in a hot-air oven and preserved.

The staining solution ready for use is then prepared as follows:

Take .3 gm. of the residue, which is a greenish crystalline powder, and add it to 100 cc. of pure methylic alcohol (Merck's reagent). This should then be filtered and to 80 cc. of the filtrate, 20 cc. of methylic alcohol added. The solution is now ready for use.

To stain, add a few drops of this solution to the preparation to be stained and let stand for two minutes, then add enough distilled water to cause a slight metallic scum to form on the surface of the fluid; then let stand for from two to five minutes, wash the preparation in running distilled water, and examine at once or mount in Canada balsam.

The Morphology of Intracorpuseular Conjugation: Estivoautumnal Infections.—I shall describe the process in detail as seen in this variety of malaria, as here it is seen most typically. In fresh specimens of blood I have seen conjugation several times, but it should be remembered that it is but rarely that the process can be observed in such specimens. As is well known, it is very common in specimens of blood from patients suffering from estivoautumnal infections to see red cells containing two or even more ring forms of the plasmodia; such parasites, however, are not always conjugating forms, although I believe that conjugation follows sooner or later in most of them. We must admit that in some instances the two parasites develop independently, so far as we can determine, for I have seen red corpuscles containing two plasmodia at different stages of development, and this is not uncommonly observed in tertian infections. In the fresh blood, conjugation commences with the gradual approach of the two parasites until finally they lie in apposition, the ameboid processes of the adjoining segments of the rings apparently merging, so that in a few seconds there is no distinction between the parasites at the point of apposition. Gradually more and more of the protoplasm of the parasites blend until at last, usually after a period of from 10 minutes to half an hour, conjugation is complete and only one parasite can be distinguished in the cell. Of course, in the fresh blood none of the structural elements of the parasites can be seen, so that the entire process appears to consist merely in the apposition of the plasmodia and their gradual union. It would appear as though, after the apposition of the two parasites, the limiting membrane of the organisms ruptured at the point of apposition and thus allowed the protoplasm of the two rings to unite, producing one organism.

Stained Preparations.—In stained preparations all the various stages of the process of intracorpuseular conjugation can be easily distinguished as the morphology of the parasites is thus rendered visible. In preparations stained by the method described, the ring form of the estivoautumnal parasite is seen to consist of three portions: The protoplasm, which stains blue; the chromatin mass, which stains a bright red, and the achromatic substance of the nucleus, containing the chromatin mass. The mass of chromatin is situated, before conjugation, at some portion of the periphery of the ring, generally at its most narrow part, but after conjugation is complete the united masses of chromatin lie within the ring, surrounded by the achromatic substance, outside of which lies the blue-stained protoplasm. For clearness in description, the process of intracorpuseular conjugation

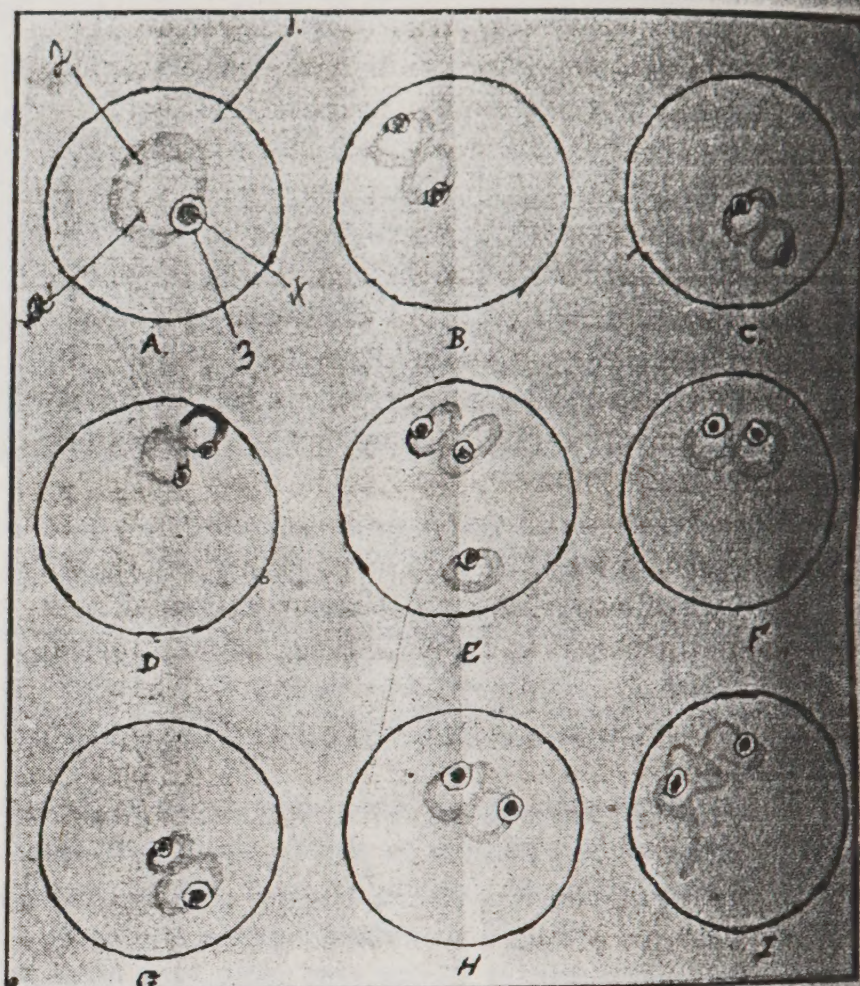


Fig. 1.—First stage of intracorpuseular conjugation (apposition of the protoplasm). A, diagram of stained estivoautumnal plasmodium: 1, red blood-corpuscle; 2, protoplasm of parasite; 3, achromatic substance; 4, chromatin. B to H, showing apposition of the protoplasm and situation of chromatin granules. E shows a conjugating pair and a single parasite. I, conjugation in tertian parasite showing ameboid process.

may be divided into three stages, but it should be remembered that there are many slight morphologic departures from the typical picture, which, so far as a clear understanding of the process is concerned, it is unnecessary to describe. In all the specimens, parasites are seen exhibiting the various stages of the process, but it is only observed when the young hyaline parasites are present.

First Stage.—The first stage of intracorpuseular conjugation consists in the apposition of two hyaline ring forms, and in stained preparations it will be observed almost invariably that apposition of the two parasites occurs at some portion of the ring remote from the chromatin masses. In other words, conjugation begins with the union of the protoplasm of the parasites, and

not by nuclear union. The chromatin masses may be situated at any portion of the individual parasite, but it is extremely rarely that they lie in apposition at this stage, and when they do, I believe that the parasites containing them are not true conjugating bodies.

Careful examination demonstrates that at the portion where the rings are in apposition there is a direct union of the protoplasm, as the blue stain is uniform at that point, and there is no evidence of the outline of the rings nor of overlapping.

I have been unable to detect any difference in the conjugating bodies as regards the staining reactions, but not infrequently one may appear slightly larger than the other, the reason for which I have already mentioned. The chromatin masses are always of the same size at this stage and stain a brilliant red, and both rings are distinct, except at the point of apposition. The various appearances presented are represented diagrammatically in Fig. 1.

Second Stage.—The second stage of this form of conjugation results in the complete union of the protoplasm of the two parasites, thus making the distinction into two ring forms impossible. By this union, the two chromatin granules become situated in the protoplasm of one parasite, and they may be placed opposite one another or at any portion of the periphery of the parasite, sometimes almost in apposition. The complete union

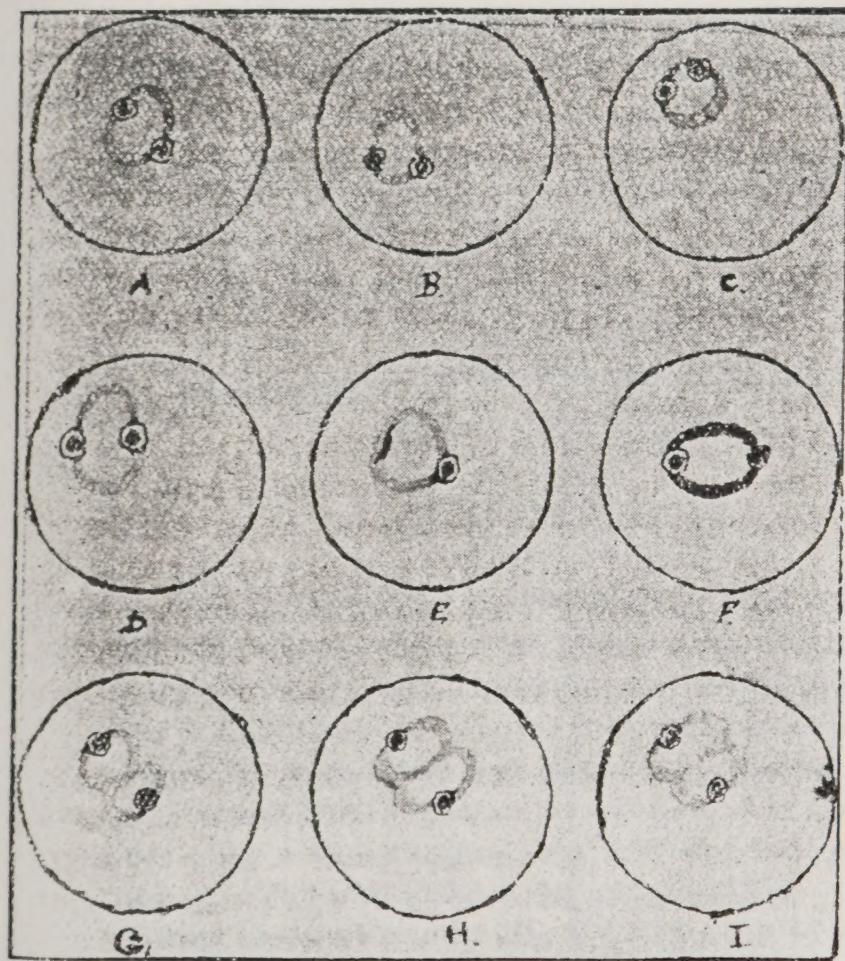


Fig. 2.—Second stage of intracorpuseular conjugation (union of the protoplasm). A to F, illustration of protoplasmic union, showing situation of the chromatin clumps and the loss of distinction into two rings. G to I, illustrating the union of the protoplasm of the two parasites leading to the formation of one ring by attenuation of a portion of the protoplasm when apposition occurs.

of the protoplasm of the plasmodia results in a more or less perfect ring-shaped organism, slightly larger than either of the original parasites, containing two masses of chromatin surrounded by achromatic substance, which are situated at some portion of the periphery of

the ring, but not in apposition. Various stages of the union of the protoplasm of the two parasites may be observed, but the most common method appears to be by a gradual disintegration of the central portion of the adjoining segments of the rings until only a thin strand

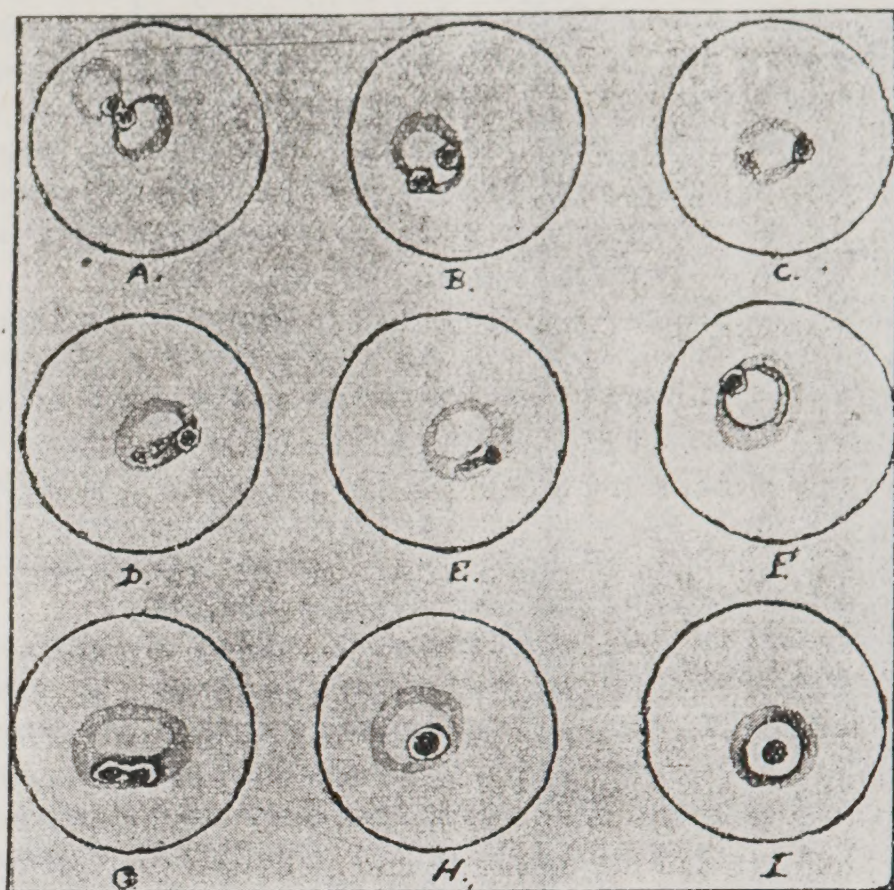


Fig. 3.—Third stage of intracorpuseular conjugation (nuclear union). A, false conjugation; primary apposition of chromatin. B, showing situation of chromatin clumps near together. C, D, E, various stages in the approach and union of the chromatin clumps, when situated far apart. F, showing the spreading out of the chromatin of one clump in process of reaching and uniting with the others. G, union when chromatin clumps are near together. H and I, situation of the chromatin clump after union is complete.

of protoplasm stretches across what eventually becomes one ring-shaped organism. This, of course, occurs upon the disintegration of the minute portion of protoplasm. This method of union is represented in Fig. 2.

It will be seen that after this union is complete, the clumps of chromatin may be opposite one another, or situated at irregular intervals in the protoplasm of the parasite.

Third Stage.—This stage of the process is characterized by the union of the chromatin masses, with the apparent exclusion, in many instances, of a very minute granule of chromatin before union is complete.

In many specimens, it will be noticed that the chromatin in one of the clumps instead of being collected in a compact mass, is separated into very fine filaments or granules, and distributed over a segment of the ring, thus bringing it nearer to the other mass of chromatin, which is compact in formation. This is the first stage of nuclear union, and is seen only when the chromatin masses are opposite one another or some distance apart. In rare instances, both masses of chromatin show thinning into filaments or granules.

In cases in which the chromatin clumps are near together, union is effected by a lengthening of the masses until they meet, while in the first instance mentioned, the thinned mass of chromatin apparently travels around the intervening segments of the ring

until it meets the other mass and unites with it. This movement is probably brought about by protoplasmic currents, which carry the chromatin in the desired direction.

After the union of the two masses of chromatin, which occurs almost always at some portion of the periphery of the organism, the chromatin takes up a position within the ring, generally perfectly central, and is surrounded by a well-defined achromatic zone, and this again by the protoplasm. The appearance of the chromatin after union is that of a very compact circular mass, which stains a brilliant red by the staining method described.

In many specimens a very small, dark-red grain of chromatin may be seen lying at some distance from the nearly united masses of chromatin, and it appears to have been thrown off from one of the chromatin clumps. Whether or not this is an essential part of the process of nuclear union, as it is in many of the protozoa, I have been unable to determine. However, it is by no means always present.

In all stained specimens showing the process of intracorpuseular conjugation the stages described can be easily recognized, and may, I believe, be interpreted as follows: The conjugating parasites unite at some portion of the peripheral protoplasm, the two chromatin granules remaining separate; gradually the protoplasm of the organisms unites, this union being followed by the union of the chromatin masses and the achromatic substance of the nuclei, and thus conjugation is completed.

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- ² Clinical Pathology of the Blood, 1903, p. 454.
- ³ Jour. of Exper. Medicine, Vol. v, No. 5, March, 1901.
- ⁴ Loc. cit.
- ⁵ Loc. cit.
- ⁶ Clinical Pathology of the Blood, 1903, p. 454.

[To be concluded.]

WHAT IS A POISON?

BY

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We have learned by observation that there are substances which, when swallowed in minute amounts, are capable of seriously injuring or killing living beings. Such substances are known to everybody as poisons. We know by experience that there are substances essential to life and health that can be swallowed in large amounts not only without injury, but with decided benefit to the taker. These constitute our chief articles of diet. Between these extremes lies a third class in which large amounts are dangerous, but in which small quantities are comparatively harmless. Of these some are used as foods, some as condiments, some as beverages, some as medicines, and there are people who call many of them poisons. This last group is the cause of much conflict of opinion. The growing tendency to call everything a poison that self-interest or bias wishes to cast opprobrium upon is a noteworthy trait of human frailty. The therapeutic nihilist classifies all drugs used in medicine as poisons. He thus seeks to carry to the minds of others the implication that they cannot be beneficial but must be inju-

rious. Prohibitionists classify all alcoholic beverages as poisons, and some of them go so far in their condemnation as to declare that alcohol is so dangerous that it is sinful to prescribe it as a remedy. The opponents of tobacco are quite sure that it is a dangerous poison and that it is sapping the vitality of nations and crippling the vitality of the unborn. Antivivisectionists are positive that antitoxins are poisons, and antivivisectionists declare, with all the vigor at their command, that the vaccinator inoculates the systems of his patients with septic and other foul poisons. So-called dietetic reformers tell us that coffee and tea, salt and pepper, spices and meats, are all poisons that should be eschewed by every sensible man and woman in the world. Many of the advocates of pure food measures denounce the protection of perishable foods from decomposition and from the development of disease germs by the use of antiseptics as a poisoning of the food. They look upon borates, benzoates, and salicylates as poisons. Amid this babel of voices stand the laws of States and nations making the use of poisons in foods into misdemeanor or felonies. Druggists must comply with poison laws that forbid the sale of poisons except under certain restrictions and neither they, the judge, the jurors, the lawyer, the legislators, nor anyone else, can tell what are to be considered poisons and what innocuous substances until an accident occurs that ties them up in the meshes of the uninterpretable law. In multitudes of cases tried within our courts, medical men hazard opinions on what constitutes a poison, without due thought or study of the matter, and not thinking that they may be aiding in the establishment of a precedent on which the lives and liberties of others may hang. On the doctor's idea of what constitutes a poison may turn the decision of a judge or a juror that will bring penalties and odium on the innocent. On his ideas of what constitutes a poison may depend his treatment of some patient in his care. A false conception may make him injure instead of benefit his patient. The liberties and rights of whole nations may be shackled by false notions on this subject and the progress of science handicapped by the restrictions of legislation brought about by overzealous reformers. It is only a few centuries since that none but apothecaries dared to sell sugar, and they were compelled to dole it out in such doses as a mentally terrorized medical profession ventured to prescribe.

No one will deny that for an average man such substances as the toxalbumins, hydrocyanic acid, aconitine, atropin, strychnin, nicotin, morphin, digitalin, arsenic, and mercuric chlorid are poisonous when given to human beings in doses of a single grain or over. Some of them we know will kill an adult in doses much smaller than this. These are the kinds of substances that the majority of people think of when the word poison is mentioned in their hearing. No one ever questions the propriety of calling these by that name whether in the courts or in the streets. If we dilute any of these substances, or such of them as are used as therapeutic agents, until a teaspoonful of the mixture contains less than an average therapeutic dose, is the mixture a poison? If we use water, sugar, or starch with which to dilute, will the combination be a poison? If we take one of the most toxic of

deavor to make the operation direct, brief, and the anesthesia short, I never hurry nor have any fretting or scolding in the operating-room, and an observer would never conclude that I was operating rapidly and would generally be surprised only at the ease and quietness of the procedure and the slow advance of the clock.

The patients themselves are not generally nauseated and depressed by the short gas-ether anesthesia. They are ready to sit up as soon as they wake up, and are anxious to get out of bed for the first movement of the bowels. They never receive any stimulants or restoratives during the anesthesia, and it is most exceptional for them to receive any medicine afterward, except a laxative or carminative. Most patients are able to drink by the end of six hours, or 12 hours at the most, and after the simpler operations they rarely miss more than one or two meals before they are placed upon light diet. After many of the operations the patients dress and go about the hospital on the second or third day, and rarely remain in the hospital longer than a week.

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[Concluded from page 986.]

Tertian and Quartan Infections.—It is unnecessary to describe fully the process as it occurs in tertian and quartan infections, as the phenomena do not differ in any essential particular from those observed in estivo-autumnal infections. Conjugation is completed during the young hyaline stage, before the formation of pigment, in both forms of infection, but it does not always occur in the ring forms, for many times conjugating plasmodia are seen in which ameboid motion has distorted the ring, and bizarre-shaped parasites result. None of these conjugating organisms has shown the presence of pigment, however, and should not be confused with the pigmented parasites in which pigment is present and two masses of chromatin granules; such organisms are not conjugating bodies, but that stage of the development of the plasmodium in which the division of the chromatin takes place prior to the formation of spores.

In his contribution, Ewing speaks of the large size of the sporulating bodies which result after conjugation, but I have been unable to confirm this statement, as it would appear impossible to be sure that large sporulating bodies were due to conjugation, as the process being completed during the hyaline stage of growth, it is afterward impossible to distinguish the conjugating parasites from those in which the process is absent. While such large sporulating bodies may result from conjugation, and the probability is that they do, I have

been unable to demonstrate, to my own satisfaction, that such is the case.

From the study of the process of conjugation, the morphology of which has been briefly given, I believe that it is obvious that the process is not an accidental one but one of great importance in the human life cycle of the malarial plasmodia; the definite stages of the process which can be traced in practically every specimen of blood showing conjugation, proves that it is a vital process, and one that is in some way essential in the life history of the parasites. In order, therefore, to determine its significance it is necessary to learn the frequency of its occurrence, the class of cases in which it occurs most frequently, and whether or not these cases present any peculiar clinical phenomena. It is also necessary to review the significance of the process of conjugation in other protozoan organisms.

The Frequency of the Occurrence of Intracorpuseular Conjugation and the Clinical Types of Malaria in which It is Present.—As I have already stated, intracorpuseular conjugation is present in all three of the great classes of malaria, tertian, quartan and estivoautumnal, and occurs as frequently in one variety as another, although the process is best observed in blood from estivoautumnal cases. In order, however, to arrive at any conclusion regarding the significance of this form of conjugation, it is necessary to determine in what period of these infections it occurs, and what, if any, are the clinical phenomena produced by it. Does conjugation occur only during the initial acute attacks of malarial infection or also during recurrent and latent infections? What is its duration as regards the length of the infection, and what influence has it upon the symptomatology of the malarial disease? These questions can only be answered by a careful study of the blood in initial and recurrent attacks of malaria and latent infections. As stated, I have studied the blood with reference to intracorpuseular conjugation in several hundred cases of malaria, divided as follows: Acute initial attacks, 52; recurrent attacks, 289; latent malarial infections, 195; and the conclusions regarding the frequency of the process and its significance are based upon the data derived from these examinations.

Initial Acute Malaria.—The 52 cases of acute initial attacks of malaria were observed at Camp Stotsenburg, in the persons of soldiers and civilians located at or near that post. Tertian, quartan and estivoautumnal cases were all present, and thus a good opportunity was afforded of studying the process of conjugation in undoubted acute initial attacks of all the varieties of malaria.

As regards the frequency of conjugation in acute initial attacks, it may be stated that it is invariably present, for in every case which I have examined, examples of conjugation were seen. There appeared to be a definite relationship between the clinical symptoms and the number of the conjugating parasites, for in those cases in which the symptoms were most severe, conjugating plasmodia were most numerous. In all the cases, however, they were seen, though often when the symptoms were slight, repeated examinations of the blood had to be made before the conjugating pairs were

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demonstrated, but patient search always resulted successfully. I have never found it necessary to resort to puncture of the spleen, on the supposition that conjugation may be present in the splenic blood when not in the peripheral, as suggested by Ewing, for I have never failed to observe it in the peripheral blood in acute initial attacks of malaria.

In many acute infections, marked by severe clinical symptoms, conjugating parasites are very numerous, sometimes, especially in estivoautumnal infections, one field of the microscope showing two or more red cells containing conjugating pairs.

After the administration of quinin, conjugation cannot be observed, even though a generation of the plasmodia have escaped the drug and entered the red corpuscles, and in many instances, when spontaneous recovery has occurred, so far as the clinical symptoms are concerned, I have examined the blood repeatedly, and very carefully, and have never been able to find any examples of conjugation, even when plasmodia were present and were sporulating. This fact proves that the connection between the production of clinical symptoms and conjugation is very intimate, that conjugation is not essential to sporulation, and that its disappearance leads to spontaneous recovery.

Recurrent Malarial Attacks.—I have examined the blood in 289 cases of recurrent malarial fever, all of them being in soldiers invalided home from the Philippines to San Francisco, Cal. All of these patients had had repeated attacks of malaria, and most of them, at the time of examination, were suffering from the estivoautumnal type.

As would be expected from the frequency of conjugation in initial acute attacks and the close relation of the process to the clinical symptoms, in recurrent attacks, also, conjugation is present in the vast majority of instances, although not invariably. In a very small number of the cases, not more than 10, the most careful examination of the blood failed to show any evidence of the process, so that it is fair to presume that it is not absolutely essential to the production of clinical symptoms of malaria, although undoubtedly increasing their severity. All of the cases in which the process was absent presented very slight symptoms of the disease, while, as in the acute initial attacks, those cases presenting the most severe clinical symptoms also showed in the blood the greatest number of conjugating plasmodia. Quinin brought about their disappearance, as in the acute attacks, and they gradually disappeared in those cases in which spontaneous disappearance of the clinical symptoms occurred.

From the examination of these cases we must conclude that intracorpuseular conjugation is present in the great majority of cases of recurrent malaria, but that it is not absolutely essential to the production of clinical symptoms of the disease.

Latent Malarial Infections.—There were 195 cases of latent malarial infection examined, 120 of which were in soldiers returned from the Philippines, and 75 in native Filipino children. These latter cases are of especial interest, as they were discovered during the routine examination of the blood of the native children in the

barrios (villages) around Camp Stotsenburg. A detailed report of these examinations will be made later, but it is interesting to note that over 50% of the native children in this locality are harboring the malarial plasmodia, and are, undoubtedly, the great source of infection to the soldiers at Camp Stotsenburg.

All varieties of malarial infection, tertian, quartan and estivoautumnal, were represented in these cases.

A most interesting and important fact was discovered during the examination of these latent infections. With very rare exceptions, intracorpuseular conjugation was absent, and in those cases in which it was present, clinical symptoms appeared within 24 hours. The blood in the latent cases showed the presence of the plasmodia in various stages of development, and in many instances the entire human life cycle of the parasites was followed in individual cases, but, with the exception of six cases, conjugation was never observed.

The absence of conjugation in these latent cases, and its occurrence in acute and recurrent cases of malaria, throw a flood of light upon the significance of the process. While conjugation is neither essential to sporulation or to the production of clinical symptoms, it is essential to the maintenance of a malarial infection sufficient to cause severe symptoms, and in the vast majority of instances the severity of the symptoms is a direct index of the number of conjugating plasmodia present. The absence of intracorpuseular conjugation is absolutely essential to spontaneous recovery. These facts can only be explained by considering that this form of conjugation favors the production of more numerous sporulating parasites.

From the study of the facts as described, the following conclusions are warranted:

1. Intracorpuseular conjugation is always present in acute initial attacks of tertian, quartan, and estivoautumnal malaria, almost invariably present in recurrent attacks, and almost never present in latent infections.

2. It has, in the vast majority of cases, a direct relationship to the severity of the clinical symptoms.

3. It is not absolutely essential to sporulation or to the appearance of clinical symptoms.

4. It is essential to the maintenance of autoinfection, and its disappearance results eventually in spontaneous recovery.

The Significance of Intracorpuseular Conjugation.—In order to understand thoroughly the significance of this form of conjugation in the plasmodia of malaria, it is necessary to review briefly the process of conjugation and its significance in some of the other protozoan parasites, as only by so doing can we arrive at a clear comprehension of the fundamental facts which make this process of such great importance in the life history of many of the protozoa.

The process of conjugation, in the protozoa, is pre-eminently intended to prolong the life of the organism by rendering future generations possible, and is not a reproductive act, in the strict acceptance of that term. In those protozoa which have a life cycle in an intermediate host, however, conjugation is absolutely essential to reproduction, an example of which is found in the

malarial plasmodia, during the life cycle within the mosquito.

The process of conjugation in the protozoa was first observed and described by O. F. Müller.⁷ His work was confirmed by Balbiani,⁸ who claimed that certain of the protozoa not only reproduced by simple division but also by conjugation, the latter being a sexual act leading to the formation of the young parasites. It is to Bütschli⁹ that we owe the correct interpretation of conjugation in the protozoa. He observed that continued reproduction of many of the protozoan organisms by simple division leads eventually to the exhaustion of the capability of division, and thus to the death of the organisms. Such organisms greatly diminished in size and in vital activity, and, unless conjugation occurred, lost the power of reproduction. He, therefore, regarded the process of conjugation as intended to bring about rejuvenescence in the nearly exhausted individuals of a generation of organisms. Engelman¹⁰ confirmed the interpretation of Bütschli, and thus defines the process: "The conjugation of the infusoria does not lead to reproduction through 'eggs,' 'embryonic spores,' or any other kind of germ, but to a peculiar developmental process in the conjugating individual, which may be designated as reorganization."

In other words, conjugation is intended to bring about a restoration to former reproductive activity, this result being secured by a rejuvenescence of the vital activities of the organism.

Calkins,¹¹ in his admirable discussion of this subject, says:

If, as Minot suggested, every newly-formed organism be regarded as having a certain initial potential energy which is gradually used up in its life activities to be restored by conjugation, then the union of two cells may be interpreted as a renewal of vigor or a rejuvenescence.

The force of these views as to the need of conjugation for different species of infusoria, at least, can hardly be questioned, for, as repeatedly stated in the previous chapters, reproduction by simple division may go on for a certain number of generations, but cannot continue indefinitely, unless at certain intervals, which Maupas has shown to be more or less definite, two individuals unite in conjugation. This union, in some wholly unexplained way, imparts to each of the conjugants a renewed vitality, or in Bütschli's words, a renewal of youth, expressed by increased activity in movements and reproduction. Conjugation thus, as R. Hertwig insists, is not the beginning of a series of reproductive acts, but occurs at or near the end of such a series. Maupas' results seem to offer conclusive evidence that the absence of conjugation involves a cumulative degenerative process which ultimately ends in death. The phenomena of the so-called sexual reproduction and sex differentiation have, in all probability, grown out of this apparently fundamental requirement of living protoplasm, namely, the periodic union of two cells. . . .

The process of conjugation has been observed and studied in all classes of protozoa, in some of which it is much more highly specialized than in others. Indeed, as Calkins states, the whole process of sex differentiation can be traced by a careful study of the phenomena of this process, so far as the protozoa are concerned. —

In the *Sarcodina* it occurs, so far as is known at present, but rarely, and especially in *Arcella* and a few *Amœbæ*, I have observed it repeatedly in *Entamœba*

dysentericæ and *Entamœbæ coli*, and it is probably of much greater importance in maintaining the life of these organisms, and consequently infection, than is at present believed. In both these amœbæ conjugation consists in the permanent fusion of apparently adult organisms, of about the same size, which is probably followed by fusion of the nucleus. In the *Mastigophora* conjugation has been very thoroughly studied and is comparatively frequent. In this class the formation of spores is often preceded by conjugation. The process is common among some of the monads, and consists in the fusion of adult organisms. In the *Cercomonads* conjugation commences with the fusion, at first, of the protoplasm, the flagellas being retracted within the organism; eventually the nuclei unite and a cyst is formed within which the young spores develop, and are liberated by rupture of the cyst wall. This method of conjugation is closely analogous to that occurring in the malarial plasmodiums, which I have described. Strong has also seen conjugation in *Balantidium coli*, consisting in the fusion of adult organisms.

In the *Sporozoa*, conjugation is very highly developed and in this class of the protozoa sexual differentiation is often apparent, the conjugating organisms varying in size and even in structural details. In the *Hæmosporidiida*, *Gregarinida*, and *Coccidiida*, conjugation has been carefully studied by Siedlecki and Schaudinn, who have contributed the most valuable additions to our knowledge of this process in the protozoa. In the *Coccidiida*, an asexual and sexual cycle of development is present, conjugation being a developmental necessity in the latter. The same is true of the plasmodia of malaria, which have an asexual cycle of development in man and a sexual cycle in the mosquito. In the latter conjugation is absolutely essential to sporulation, but here the conjugating organisms are sexually differentiated, while in the human cycle, conjugation is not essential to sporulation and the conjugants cannot be sexually differentiated. In fact, the two cycles of the plasmodia of malaria illustrate conjugation in its simplest and most highly developed phase.

Having thus briefly reviewed the phenomena of conjugation in the most important classes of the protozoa, and its significance, we are prepared to interpret its significance in the human life cycle of the malarial plasmodiums.

These parasites reproduce in man by spore formation, and it has been shown that intracorpuseular conjugation is not essential to the formation of spores, for in latent malaria the entire cycle of development can be followed when conjugation is absent. Continued reproduction, however, will end ultimately in exhaustion, as in other organisms, and it is prevented by conjugation after a certain number of generations of the plasmodia have been produced by asexual reproduction. Intracorpuseular conjugation in the plasmodia of malaria results in a rejuvenescence of the nearly exhausted individuals and renewed activity in reproduction, and thus it is essential to the maintenance of the malarial infection.

Considered in this way, it is not difficult to reconcile the clinical and microscopic findings, so far as conjugation is concerned.

In initial acute malarial infections, conjugating parasites, as I have said, can always be demonstrated in the blood. In such cases, sporulating parasites are present in comparatively large numbers, thus proving that sporulation is favored, as in cases in which conjugation is not present, sporulating bodies are very scanty, and there are few or no clinical symptoms present. This fact can only be interpreted as meaning that the presence of conjugation has produced increased activity in reproduction.

The same interpretation may be placed upon the occurrence of conjugation in the recurrent malarial infections, and the deduction is proved by the fact that conjugation disappears during spontaneous recovery, and reappears just before the recurrence of the clinical symptoms.

In the latent infections, conjugation is, practically, always absent, the only cases in which it was found being a few which developed, within 24 hours, the clinical symptoms of malaria, at which time conjugating parasites were found. Although the process is not essential to the production of symptoms, as shown by the fact that conjugating parasites could not be demonstrated in a few of the recurrent cases, there can be no doubt but that conjugation plays a very important part in the production of the clinical symptoms by favoring the sporulation of the plasmodia.

From the data obtained from these studies I believe that the following conclusion can be drawn: After infection of man by the bite of the infected mosquito, the plasmodia reproduce asexually for a number of generations (this is proved by the examination of the latent cases) without producing symptoms, or, at most, very slight symptoms of malaria. After reproduction has gone on in this manner for some time, the plasmodia become exhausted, and intracorpuseular conjugation commences, thus increasing the reproductive ability, and, in the majority of instances, leading to the production of the clinical symptoms. In those cases in which spontaneous recovery occurs, conjugation for some unknown reason ceases, and recovery ensues.

The conclusion arrived at regarding intracorpuseular conjugation is, I believe, substantiated by the facts which have been given, and is the one which best explains the occurrence of the process in acute and recurrent malarial attacks, its absence in latent infections, and its disappearance during spontaneous recovery. It is a process intended to preserve the reproductive power of the plasmodia by "rejuvenescence," thus preventing their death, and, what is of most interest to the physician, maintaining the malarial infection.

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- ² Jour. de la Physiol., Vol. iv, pp. 102-30, 194-220, 431-48, 465-520.
- ³ In Bronn's "Classen und Ordnungen des Thierreichs," Leipzig, 1883-1888.
- ⁴ Morphologisches Jahrbuch., i, pp. 573-635.
- ⁵ The Protozoa, New York, 1901, p. 212.

A Medical Hotel.—According to the *Riforma Medica* there is to be built in Rome a medical hotel which will be equipped so as to furnish the best medical care and attendance.

SUBJECTIVE OZENA.

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The existence of ozena, in its more ordinary varieties, always constitutes a physical evil of sufficient magnitude to make it important, and, in most cases, so obstinately troublesome in regard to curative treatment as to give it a very special clinical interest. The term ozena has been pretty familiar in medical literature since, at least, the date of the publication of the professional writings of Celsus. The *ὀζαινα* (from *ὀζειν*, to emit a disagreeable odor, to stink) of that Father of Medicine was very fully recognized by Galen; a fact which necessarily secured its place in the nosology of every medical writer down through the middle ages. Under the additional (or synonymous) appellations of *dysodia* and *factor narium* it received a prominent share of attention from the hands of all the "authorities." Rhazes, Avicenna, Fernellus, Forestus, Fabricius, Scultetus, Riverius, etc., treated its claims with unmistakable deference. All regarded it, too, as a condition in which an offensive odor was due to local ulceration productive of a foul discharge. No special modification of nomenclature or pathology—so far as I have been able to ascertain—arose in this connection till the appearance of the epoch-making nosology of Sauvages, who, after defining *ozæna dysodia* in concordance with the orthodox views of its pathology, proceeded to add: "La puanteur de la matière ichoreuse qui sort des narines, n'est pas un signe certain de la présence de l'ulcère, puisqu'on voit s'écouler des narines une humeur fétide, qui n'est cependant point purulente, et qui n'est autre chose que du mucus corrompu par son séjour, à cause de l'ouverture étroite des narines." Since that date, at least, the existence of a malodorous nasal discharge without necessary coexistence of an unhealthy solution of continuity of the mucous membrane has been universally acknowledged. The characteristically Gallic expressiveness of the term *punaisie*, by which it is known in semiscientific and popular French literature, has been derived—as need hardly be explained—from the supposed resemblance of the odor of the discharge to that emitted by a recently-crushed specimen of a certain loathsome insect. The tendency to classify and arrange with increasing precision of detail characterized the encyclopedic movement of the latter part of the eighteenth century, and was continued directly into the nineteenth. In accordance with this spirit, we find J. Frank making three divisions of ozena: (1) According to the seat of the local lesion—nasal fossas proper, frontal sinus, or maxillary sinus; (2) according to the nature of the disease—traumatic, catarrhal, metastatic, scorbutic, scrofulous, arthritic, venereal, carcinomatous, etc.; (3) according to the condition of the affected parts—simple and complicated, though complications including in a fair proportion of cases such lesions as caries of one or more of the bones which enter into the formation of the osseous framework of the nasal fossas. In classifying the circumstances which—directly or remotely—have been found to give rise to ozena, Piorry arranged these under five heads: (1) The simple



